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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
C07B 59/00, C07D 309/40
C07F 5/00

A1

(11) International Publication Number: WO 91/14664

(43) International Publication Date: 3 October 1991 (03.10.91)

(21) International Application Number: PCT/GB91/00441

(22) International Filing Date: 22 March 1991 (22.03.91)

(30) Priority data:
9006522.8 23 March 1990 (23.03.90) GB

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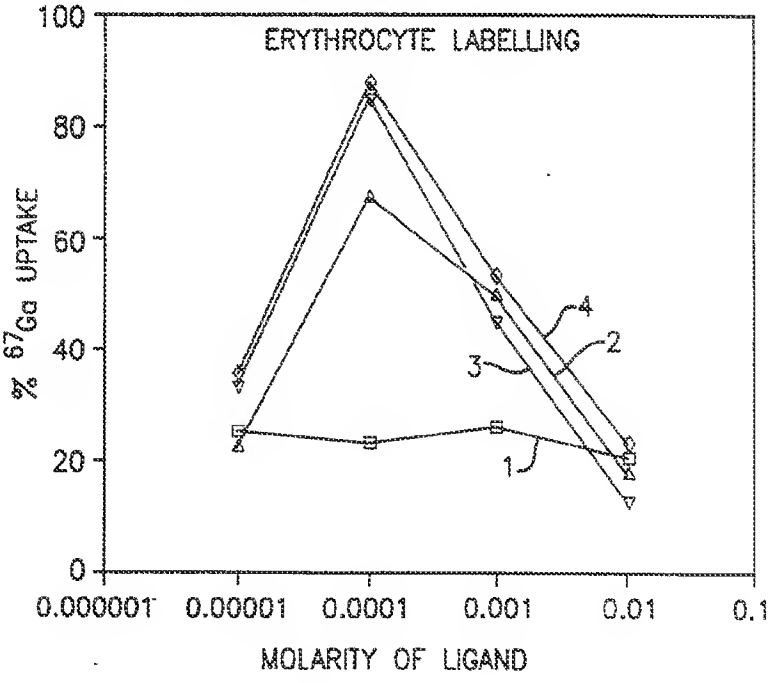
(81) Designated States: AT (European patent), BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US.

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: METAL COMPLEXES

(57) Abstract

Neutral 3:1 ligand:metal (III) complexes, in which the trivalent metal cation is a radioactive isotope of indium or gallium and each ligand is separately provided by a compound being: (1) a 3-hydroxy-4-pyrone in which one or more of the hydrogen atoms attached to ring carbon atoms is replaced by an aliphatic hydrocarbon group of one to six carbon atoms or such a group substituted by one or more groups selected from fluoro, hydroxy and aliphatic hydrocarbyloxy groups but excluding 3-hydroxy-2-methyl-4-pyrone; or (2) 3-hydroxypyridin-2-one or a 3-hydroxypyridin-2-one in which the hydrogen atom attached to the nitrogen atom is replaced by an aliphatic acyl group, by an aliphatic hydrocarbon group of 1 to 6 carbon atoms, or by an aliphatic hydrocarbon group substituted by one or more substituents selected from aliphatic acyl, alkoxy, cycloalkoxy, aliphatic amide, aliphatic ester, halogen and hydroxy groups and optionally, in which one or more of the hydrogen atoms attached to ring carbon atoms is replaced by one of said substituents, by an aliphatic hydrocarbon group of 1 to 6 carbon atoms, or by an aliphatic hydrocarbon group substituted by an alkoxy, cycloalkoxy, aliphatic ester or hydroxy group or by one or more halogen groups; are of value in therapy and particularly in diagnosis, especially in the context of cell labelling.



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METAL COMPLEXES

This invention relates to novel radioactive metal complexes and to their use in therapy and particularly diagnosis, especially in the context of cell labelling.

Radioactive indium and gallium when labelled to blood cells have been extensively used in diagnostic methods in the clinical setting. The labelling of red cells has been used as a method of splenic imaging or determination of red cell mass, platelet labelling has been used for studies on platelet survival and platelet kinetics, while white cell labelling has been used to localise inflammatory foci in a variety of clinical conditions. In a more general sense the efficient labelling of blood cells has wide implications in the diagnosis of any disorder where haemagglutination may occur, e.g. atherosclerosis, thrombocytopenia, focal sepsis and in the location of tumour and metastatic tissues.

Radioactive indium and gallium are generally used in the form of metal complexes. However, many of the agents currently used to chelate these metals are toxic to the cells, e.g. oxine (8-hydroxyquinoline) and tropolone which are two of the agents which are widely used. We have now found that cell labelling with radioactive indium and gallium may be carried out more effectively using an alternative group of chelators.

Accordingly the present invention comprises a neutral 3:1 ligand:metal(III) complex, in which the trivalent metal cation is a radioactive isotope of indium or gallium and each ligand is separately provided by a compound being:

(1) a 3-hydroxy-4-pyrone in which one or more of the hydrogen atoms attached to ring carbon atoms are replaced by an aliphatic hydrocarbon group of one to six carbon atoms or such a group substituted by one or more groups selected from fluoro, hydroxy and aliphatic hydrocarbyloxy groups but excluding 3-hydroxy-2-methyl-4-pyrone; or

(2) 3-hydroxypyridin-2-one or a 3-hydroxypyridin-2-one in which the hydrogen atom attached to the nitrogen atom is replaced by an aliphatic acyl group, by an aliphatic hydrocarbon group of 1 to 6 carbon atoms, or by an aliphatic hydrocarbon group substituted by one or more substituents selected from aliphatic acyl, alkoxy, cycloalkoxy, aliphatic amide, aliphatic ester, halogen and hydroxy groups and, optionally, in which one or more of the hydrogen atoms attached to ring carbon atoms are replaced by one of said substituents, by an aliphatic hydrocarbon group of 1 to 6 carbon atoms, or by an aliphatic hydrocarbon group substituted by an alkoxy, cycloalkoxy, aliphatic ester or hydroxy group or by one or more halogen groups.

The use in the treatment of iron deficiency anaemia of iron complexes containing certain of the ligands present in the metal complexes of the present invention has been described in UK Patents Nos. 2,117,766, 2,128,998 and 2,136,806. In 2,117,766 and 2,136,806 an alternative mode of nomenclature is used in which the 3-hydroxypyridin-2-ones are referred to as 3-hydroxypyridin-2-ones. However, both designations have the same meaning indicating a ring system as shown hereinafter in formula (V).

However, the suitability of a ligand for complexing with one metal to provide a complex for use in one therapeutic context is no indication of its value for complexing with a different metal to provide a complex for use in a different therapeutic or diagnostic context. In particular, as regards the primary, diagnostic use of the complexes of the present invention, it will be appreciated that the problem to be solved in the present application is entirely different from that with the iron complexes. With the iron complexes the ligands are required to release iron which then reassociates with apotransferrin. With the indium and gallium complexes the complexes are required to bind to the cell and emit a signal.

The different requirements of the two uses of the ligands are illustrated by recent papers relating to certain selected 3-hydroxy-4-pyrones and 3-hydroxypyridin-4-ones but not to 3-hydroxypyridin-2-ones. Thus, Finnegan et al, Inorg. Chem., 1987, 26, 2171-2176 and Matsguke et al, Inorg. Chem., 1988, 27, 3935-3939 characterise various indium and gallium complexes. These studies suggest that 3-hydroxy-4-pyrone and 3-hydroxy-2-methyl-4-pyrone, as well as certain 3-hydroxypyridin-4-ones may be of use in chelating these metals. However, although 3-hydroxy-2-methyl-4-pyrone (maltol) is one of the preferred ligands for use in the iron complexes of U.K. Patent No. 2,128,998 it is inferior to other substituted 3-hydroxy-4-pyrones for use in indium and gallium complexes as will be illustrated hereinafter.

Preferred radioactive isotopes are the isotopes which emit X-rays or positrons. Specific isotopes which are preferred, are for example the indium radioactive isotopes 111 and 113, particularly 111, and the gallium isotopes 66, 67, 68 and 72, particularly 67 and 68.

Indium 111 is particularly suitable for use in the present invention as it decays by emitting γ radiation of 171 and 245 keV and has a half life of 67.2 hours. Similarly Ga 67 is particularly suitable as it decays by emitting γ radiation of 185 and 300 keV and has a half life of 78.26 hours. Gallium 66 and 68 are suitable positron emitters.

The metal(III) complexes of use in the present invention contain indium and gallium in the trivalent form. The metal(III) complexes are neutral, i.e. there being an internal balance of charges between the metal(III) cation and the ligand(s) bound covalently thereto without the necessity for the presence of a non-covalently bound ion or ions to achieve such balance. Thus, these ligands are monobasic and bidentate, being formed through the loss of a proton from the hydroxy group of the ligand forming compound ($OH \longrightarrow O^-$). Metal(III) complexes containing a 3:1 proportion of monobasic, bidentate ligand:metal(III) are therefore neutral.

The complexes according to the present invention may contain various different combinations of three ligands of the types (1) and (2). Thus all three ligands within the complex may be chosen from type (1), or all three from type (2), although optionally differing from within these types. Alternatively the three ligands may be chosen from both types in a 2:1 ratio, i.e. either two ligands derived from the same or two different 3-hydroxy-4-pyrones with one 3-hydroxypyridin-2-one ligand or two ligands derived from the same or two different 3-hydroxypyridin-2-ones with one 3-hydroxy-4-pyrone ligand. However, complexes of particular interest are those containing three ligands of type (2) or particularly of type (1), these ligands conveniently being identical.

As regards the 3-hydroxy-4-pyrone ligands of type (1), these 3-hydroxy-4-pyrones may carry more than one type of substituent group but substitution by one rather than two or three groups is preferred except that substitution by two groups is of interest when at least one of them is an aliphatic hydrocarbon group. The term aliphatic hydrocarbon group is used herein to include both acyclic and cyclic groups which may be unsaturated or saturated, the acyclic groups having a branched chain or especially a straight chain. Groups of from 2 to 6 carbon atoms and particularly 3 to 5 carbon atoms are of most interest except that methyl groups can be of interest when an additional substituent is also present. Saturated aliphatic hydrocarbon groups are preferred, these being either cyclic groups such as the cycloalkyl groups cyclopropyl and cyclohexyl or, more particularly, acyclic groups such as the alkyl groups methyl and ethyl and especially propyl, butyl and pentyl (and their branched chain analogues). Similar observations and preferences apply to the aliphatic hydrocarbyl groups present in substituents which are aliphatic hydrocarbon groups substituted by aliphatic hydrocarbyloxy groups with the exception that the latter may sometimes be a methoxy group. Moreover, the aliphatic hydrocarbon group which is substituted by an aliphatic hydrocarbyloxy group may often be a methyl group.

In this specification the term alkyl includes both straight and branched chain groups but references to individual alkyl groups such as "propyl" are specific for the straight chain group only. An analogous convention applies to other generic terms.

When the 3-hydroxy-4-pyrone contains a substituted aliphatic hydrocarbon group that group preferably contains only one type of substituent among fluoro, hydroxy and aliphatic hydrocarbyloxy groups.

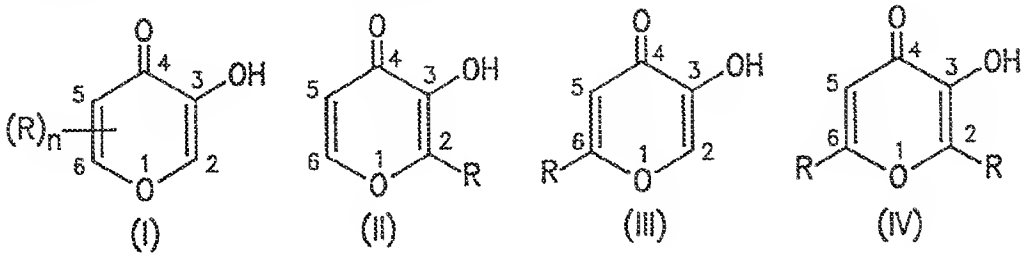
As an alternative, or in addition to, the use of aliphatic hydrocarbon groups as substituents in the 3-hydroxy-4-pyrones, fluoro substituted aliphatic hydrocarbon groups may be used, for example any of the general types of or specific aliphatic hydrocarbon groups as described hereinbefore but substituted by one or more fluoro groups, for example up to five, nine or ten of such groups. Fluoro substituted aliphatic hydrocarbon groups preferably contain one, three or five fluoro atoms, specific examples of such groups being a 1,1,2,2,2-pentafluoroethyl and 3,3,3-trifluoropropyl group.

As a further alternative, or in addition to, the use of aliphatic hydrocarbon groups as substituents in the 3-hydroxy-4-pyrones hydrocarbyloxy substituted aliphatic hydrocarbon groups may be used, or to a lesser extent hydroxy substituted aliphatic hydrocarbon groups, particularly a substituted methyl group or another aliphatic hydrocarbon group substituted on the carbon atom of the aliphatic group which is attached to the pyrone ring. When the substituted aliphatic hydrocarbon groups contain substituents other than fluoro there is usually only one aliphatic hydrocarbyloxy or hydroxy group present.

Substitutions at the 6 or especially the 2 position, and sometimes at both, are of most interest although, when the ring is substituted by the larger substituent groups, there may be an advantage in avoiding substitution on a carbon atom alpha to the $-\overset{\overset{O}{\|}}{C}-\overset{|}{C}<_{OH}$ system. This system is involved in the complexing with indium or gallium and the close proximity of one of the larger

aliphatic hydrocarbon groups may lead to steric effects which inhibit complex formation.

Examples of hydroxypyrones providing ligands which may be used in complexes according to the present invention have the formula (I), specific hydroxypyrones of interest having the formulae (II), (III) and (IV):



(I) (II) (III) (IV)

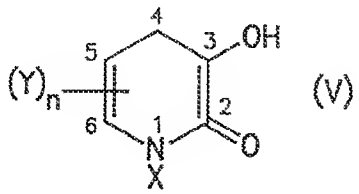
in which each R separately (subject to the exclusion of the compound 3-hydroxy-2-methyl-4-pyrone,) is an alkyl or cycloalkyl group, or such a group substituted by three or five fluoro groups, by an alkoxy group or by a hydroxy group, for example ethyl, propyl, isopropyl, butyl or pentyl, or a fluoro substituted derivative thereof containing three or five fluorine atoms, particularly 1,1,2,2,2-pentafluoroethyl or 3,3,3-trifluoropropyl, or a C_{1-4} alkoxy substituted derivative thereof, particularly methoxymethyl, ethoxymethyl, propoxymethyl or butoxymethyl, and n is 1, 2 or 3. Preferred compounds are those which are 2-alkyl, 2,6-dialkyl, 2-fluoroalkyl, 2-alkoxyalkyl, 2-hydroxyalkyl or 6-alkyl-2-hydroxy- alkyl substituted.

Among these compounds 2-ethyl-3-hydroxy-4- pyrone and its 2-(1'-methylethyl), 2-propyl, 2-butyl and 2-pentyl analogues [ethyl-maltol, isopropyl-maltol, propyl-maltol, butyl-maltol and pentyl-maltol; II, R = C_2H_5, $CH(CH_3)_2$, $(CH_2)_2CH_3$, $(CH_2)_3CH_3$ or $(CH_2)_3CH_3$] are of particular interest as are its 1',1',2',2',2'-pentafluoroethyl and 3',3',3'-trifluoropropyl analogues [II, R = CF_2CF_3 and R = $CH_2CH_2CF_3$].

Also of particular interest are the compounds of formula (III) in which R is an alkoxymethyl group and the compounds of formula (IV) in which the group R at the 6-position is an alkyl group and that at the 2-position is an alkyl or 1-hydroxyalkyl group, for example the compounds 3-hydroxy-6-methoxymethyl-4-pyrone,

6-ethoxymethyl-3-hydroxy-4-pyrone, 3-hydroxy-6-propoxymethyl-4-pyrone, 6-butoxymethyl-3-hydroxy-4-pyrone, 2-ethyl-3-hydroxy-6-methyl-4-pyrone, 3-hydroxy-6-methyl-2-propyl-4-pyrone, 2-butyl-3-hydroxy-6-methyl-4-pyrone, 3-hydroxy-2-hydroxymethyl-6-methyl-4-pyrone, 3-hydroxy-2-(1-hydroxyethyl)-6-methyl-4-pyrone, 3-hydroxy-2-(1-hydroxypropyl)-6-methyl-4-pyrone and 3-hydroxy-2-(1-hydroxybutyl)-6-methyl-4-pyrone.

As regards the 3-hydroxypyridin-2-one ligands of type (2), these may be derived from hydroxypyridinones of the type described in UK Patent No. 2,117,766 (corresponding to US Patent No. 4,550,101 and Japanese Patent Application No. 83/049677) or of the type described in UK Patent No. 2,136,806 (corresponding to US Patent No. 4,810,491 and Japanese Patent Application No. 84/057186). The former consist of a 3-hydroxypyridin-2-one in which the hydrogen atom attached to the nitrogen atom is replaced by an aliphatic hydrocarbon group of 1 to 6 carbon atoms and, optionally, in which one or more of the hydrogen atoms attached to ring carbon atoms are also replaced by the same or a different aliphatic hydrocarbon group of 1 to 6 carbon atoms, whilst the latter include 3-hydroxypyridin-2-ones substituted as defined under (2) hereinbefore but excluding those compounds in which the replacement of hydrogen atoms is effected only by aliphatic hydrocarbon groups, these compounds being the substituted hydroxypyridinones of the former patent. It will be noted that the 3-hydroxypyridin-2-ones may contain alkoxy and cycloalkoxy groups and that, as compared with the compounds of U.K. Patent No. 2,136,806, an aliphatic hydrocarbon group on a carbon atom of the ring, as well as one on the nitrogen atom of the ring, can be substituted by one or more halogen groups. Hydroxypyridinones providing ligands which may be used in complexes according to the present invention have the formula (V)



(V)

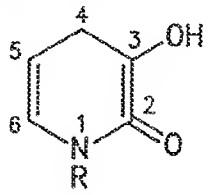
in which X and Y are substituents as defined hereinbefore as substituents on the nitrogen and carbon atoms of a 3-hydroxypyridin-2-one and n is 0, 1, 2 or 3.

Preferences as to the nature and position of the substituent groups present in the hydroxypyridinones are broadly as expressed in the aforementioned patents. Thus, substituted aliphatic hydrocarbon groups present in the hydroxypyridinones may conveniently contain 1 to 8 and particularly 3 to 6 carbon atoms and, as indicated, may carry more than one substituent group, although it is preferred that only one substituent group is present. However, the simpler hydroxypyridinones of UK Patent GB 2,118,176 containing only unsubstituted aliphatic hydrocarbon groups are of the greatest interest so that a preferred type of compound is a 3-hydroxypyridin-2-one in which the hydrogen atom attached to the nitrogen atom is replaced by an aliphatic hydrocarbon group of 1 to 6 carbon atoms and, optionally, in which one or more of the hydrogen atoms attached to ring carbon atoms are replaced by the same or a different aliphatic hydrocarbon group of 1 to 6 carbon atoms. The preferences among the aliphatic hydrocarbon groups present in these hydroxypyridinones correspond largely to those expressed hereinbefore in relation to the hydroxypyrones, with methyl groups conveniently being used for substitution on ring carbon atoms but larger alkyl and cycloalkyl groups, particularly as described for the hydroxypyrones, being of particular interest for substitution on the ring nitrogen atoms.

Substitution of the ring carbon atoms of the hydroxypyridinones is again preferably by one rather than two or three groups, for example aliphatic hydrocarbon groups, but 3-hydroxypyridin-2-ones also of particular interest are those N-substituted without any additional substituent on the ring carbon atoms.

Among the 3-hydroxypyridin-2-ones containing substituted aliphatic hydrocarbon groups those in which the aliphatic hydrocarbon group is substituted by one or more halogen groups, particularly fluoro groups, are of especial interest. The preferences for such halogen substituted aliphatic hydrocarbon

groups are largely as expressed hereinbefore in relation to the fluoro substituted aliphatic hydrocarbon groups of the hydroxypyrones. In this case however, there is a greater possibility of both an aliphatic hydrocarbon group and a halogen substituted hydrocarbon group being present in the compound, with one, for example the aliphatic hydrocarbon group, being on a carbon atom and the other, for example the halogen substituted hydrocarbon group, on the nitrogen atom. Specific hydroxypyridinones of particular interest have formula (VI),



(VI)

in which R is an alkyl or cycloalkyl group, or such a group substituted by one, three or five fluoro groups, for example ethyl, n-propyl, isopropyl, butyl, pentyl or hexyl, or a fluoro substituted derivative thereof containing three or five fluorine atoms, particularly 1,1,2,2,2-pentafluoroethyl or 3,3,3-trifluoropropyl. Among such compounds 1-ethyl-3-hydroxpyridin-2-one, 3-hydroxy-1-propylpyridin-2-one, 3-hydroxy-1(1'-methylethyl)-pyridin-2-one, 1-butyl-3-hydroxpyridin-2-one and 3-hydroxy-1-pentylpyridin-2-one are of particular interest.

Among the quite wide range of ligands described above certain ligands or combinations of ligands will be of particular value and some indication of these has already been given. One measure of the value of the preferred complexes is provided by the value of their partition coefficient (K_{part}) between n-octanol and Tris hydrochloride (20 mM, pH 7.4, Tris representing 2-amino-2-hydroxymethylpropane 1,3-diol) at 20°C, this being expressed as the ratio (concentration in organic phase)/(concentration in aqueous phase). Preferred complexes show a value of K_{part} for each ligand providing compound from 2 to 50, especially from 8 to 40, together with a value of K_{part} for the 3:1 indium(III) complex from 5 to 100,

especially from 30 to 70. The corresponding ranges for the 3:1 gallium(III) complexes are from 5 to 100 and 20 to 50, respectively. Whilst these ranges of K_{part} are given as a guide to preferred complexes, ligands and/or complexes that have a K_{part} value outside these ranges can still be suitable for use.

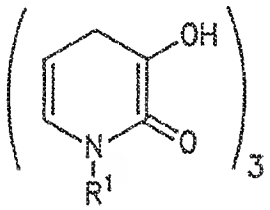
These preferences for K_{part} are reflected in the preferences for particular ligands expressed hereinbefore. Examples of specific complexes according to the present invention which are of particular interest are the indium 111 and gallium 67 complexes of hydroxypyrones and hydroxypyridinones indicated as being of especial interest, for example

$(butyl\text{-}maltol)_3$ In 111 and Ga 67

$(isopropyl\text{-}maltol)_3$ In 111 and Ga 67

$(3\text{-}hydroxy\text{-}6\text{-}propoxymethyl\text{-}4\text{-}pyrone)_3$ In 111 and Ga 67

$(6\text{-}butoxymethyl\text{-}3\text{-}hydroxy\text{-}4\text{-}pyrone)_3$ In 111 and Ga 67



In 111 where R^1 = butyl, pentyl or hexyl

The radioactive metal(III) complexes are conveniently prepared by the reaction of the hydroxypyrone and/or hydroxypyridinone ligand-providing compounds and radioactive metal ions, the latter conveniently being derived from a metal(III) salt, particularly the chloride salt. The reaction is conveniently effected in a suitable mutual solvent, usually an aqueous medium. If desired, however, an aqueous/solvent mixture may be used or an organic solvent, for example ethanol, methanol or chloroform and mixtures of these solvents together and/or with water where appropriate. In particular, methanol or especially ethanol may be used where it is desired to effect the separation of at least one major part of a by-product such as sodium chloride by precipitation whilst the indium or gallium complex is retained in solution.

Reaction is generally rapid and will usually have proceeded substantially to completion at 20°C in a few minutes. The complexes of the present invention are often used in solution but, where desired, may be prepared in solid form by evaporation of the reaction mixture and freeze drying, followed by crystallization from a suitable solvent if further purification is required.

It will be appreciated that the nature of the metal complex obtained by the reaction of the ligand-providing compound(s) and metal ions will depend both on the proportion of these reactants and upon the pH of the reaction mixture. Thus for the production of the 3:1 ligand:metal(III) complex the hydroxypyrone and/or hydroxypyridinone ligand-providing compound(s) and the metal salt are conveniently mixed in solution in at least a 3:1 molar proportion and the pH adjusted to a value in the range 6-9, for example 7 or 8. If a similar excess of hydroxypyrone and/or hydroxypyridinone:metal(III) is employed but no adjustment is made of the acidic pH which results in the admixture of the hydroxypyrone or hydroxypyridinone and a metal salt such as a metal(III) chloride, then a mixture of the 2:1 and 1:1 ligand:metal(III) complexes will instead be obtained. To ensure that all of the radioactive metal cations are combined with ligand in complex form the ligand-providing compound:metal(III) ratio may conveniently be greater than 3:1, for example being 100:1 or up to 1000:1. However, providing the ratio is 3:1 or more, and the previous requirements on reaction conditions are observed, the product will consist essentially of the 3:1 ligand:metal(III) complex. In some instances it will be preferable to incorporate some "cold" non-radioactive metal with the radioactive metal when forming the ligand:metal complex, the amount of ligand-providing compound(s) being adjusted accordingly. This will result in some non-radioactive complex being administered in combination with radioactive complex.

It will be appreciated that when the complex contains different ligands the ligand-providing compounds will conveniently be used in a 2:1 or 1:1 ratio, as desired, although with complexes in which the ligands are heterogeneous a mixture of different 3:1 complexes will always result even when a 2:1 ratio is employed. For this reason complexes in which the ligands are homogeneous are preferred.

The radioactive indium or gallium cation is preferably obtained from its chloride salt. The amount of radioactivity required in the complex is often within the range 10 to 60 mCi/µg, but will ultimately depend on the proposed use of the complex as described hereinafter.

The present invention thus further includes a process for the preparation of a neutral 3:1 ligand:metal(III) complex of a substituted 3-hydroxy-4-pyrone or 3-hydroxypyridin-2-one as defined hereinbefore which comprises reacting the ligand providing compound or compounds with the trivalent cation of the indium or gallium radioactive isotope.

Certain substituted 3-hydroxy-4-pyrones, including 2-trifluoromethyl-3-hydroxy-4-pyrone, are commercially available. For the preparation of substituted hydroxypyrones one convenient starting material consists of 3-hydroxy-4-pyrone which is readily obtainable by decarboxylation of 2,6-dicarboxy-3-hydroxy-4-pyrone (meconic acid). Thus, for example, 3-hydroxy-4-pyrone may be reacted with an aldehyde to insert a 1-hydroxyalkyl group at the 2-position, which group may then be reduced to produce a 2-alkyl-3-hydroxy-4-pyrone. The preparation of 2-ethyl-3-hydroxy-4-pyrone, etc., by this route is described in the published US Application Serial No. 310,141 (series of 1960) referred to in U.S. Patent No. 3,376,317.

The preparation of other substituted 3-hydroxy-4-pyrones may be exemplified as follows. The hydrocarbyloxyalkyl substituted 3-hydroxy-4-pyrones may be prepared from 3-hydroxy-6-hydroxymethyl-4-pyrone (kojic acid) by protecting the 3-hydroxy group, for example as a benzyloxy group, and reacting the protected compound

with the corresponding alkyl halide, for example in dimethylformamide using sodium hydride. Deprotection is then effected, for example a benzyloxy group being converted to hydroxy using concentrated hydrochloric acid, and the end product isolated. Using this method the novel compounds 3-hydroxy-6-methoxymethyl-4-pyrone, 6-ethoxymethyl-3-hydroxy-4-pyrone, 3-hydroxy-6-propoxymethyl-4-pyrone and 6-butoxymethyl-3-hydroxy-4-pyrone have been prepared. Additionally 3-hydroxy-4-pyrones wherein two of the hydrogen atoms attached to the ring carbon atoms have been substituted may also be prepared from kojic acid. For example, kojic acid is converted to the chloro analogue using thionyl chloride. This is then reacted with zinc dust and hydrochloric acid to form 3-hydroxy-6-methyl-4-pyrone. This in turn is reacted with the appropriate aldehyde to produce the corresponding 2-hydroxyalkyl-3-hydroxy-6-methyl-4-pyrone. Further reaction with zinc/hydrochloric acid yields the corresponding 2-alkyl-3-hydroxy-6-methyl-4-pyrone. Using this method the novel compounds 2-ethyl-3-hydroxy-6-methyl-4-pyrone, 3-hydroxy-6-methyl-2-propyl-4-pyrone, 2-butyl-3-hydroxy-6-methyl-4-pyrone, 2-hydroxymethyl-6-methyl-4-pyrone, 3-hydroxy-2-hydroxyethyl-6-methyl-4-pyrone, 3-hydroxy-2-hydroxypropyl-6-methyl-4-pyrone and 3-hydroxy-2-hydroxybutyl-6-methyl-4-pyrone have been prepared.

The present invention thus includes a process for the preparation of a 3-hydroxy-4-pyrone in which one or more of the hydrogen atoms attached to the ring carbon atoms is replaced by an aliphatic hydrocarbon group of one to six carbon atoms substituted by an aliphatic hydrocarbyloxy group of one to six carbon atoms, and one or more other hydrogen atoms are optionally replaced by an aliphatic hydrocarbon group of one to six carbon atoms, which comprises reacting an intermediate, in which the 3-hydroxy group is protected and the or each aliphatic hydrocarbon group substituted by an aliphatic hydrocarbyloxy group is replaced by the corresponding hydroxy substituted aliphatic hydrocarbon group, with an aliphatic hydrocarbyl halide containing the corresponding aliphatic hydrocarbyl group and then deprotecting the 3-hydroxy group.

The present invention further includes a process for the preparation of a 3-hydroxy-6-methyl-4-pyrone substituted at the 2-position by a C_{1-6} aliphatic hydrocarbon group or such a group substituted by a hydroxy group but excluding 3-hydroxy-2,6-dimethyl-4-pyrone which comprises (a) reacting 3-hydroxy-6-methyl-4-pyrone with the corresponding formyl substituted aliphatic hydrocarbon to provide a 3-hydroxy-6-methyl-4-pyrone substituted at the 2-position by a C_{1-6} aliphatic hydrocarbon group itself substituted at the 1-position by a hydroxy group or (b) effecting step (a) and then reducing said hydroxy substituted C_{1-6} aliphatic hydrocarbon group to provide a 3-hydroxy-6-methyl-4-pyrone substituted at the 2-position by a C_{1-6} aliphatic hydrocarbon group.

Certain of the substituted 3-hydroxy-4-pyrone intermediates are novel compounds and fall within the scope of the present invention.

The 3-hydroxypyridin-2-ones may be prepared by procedures described in U.K. Patents Nos. 2,117,766 and 2,136,806, and by variants thereon.

It will be appreciated that these are not the only routes available to these compounds and their indium and gallium(III) complexes and that various alternatives may be used as will be apparent to those skilled in the art.

The radiolabelled metal(III) complexes of the invention are of particular value in diagnosis when used as labels for blood cells. This requires <u>in vitro</u> labelling of the desired blood cells which are then injected back into the patient. The labelled cells will circulate and their location can conveniently be monitored by use of a gamma scintillation camera or PET scan. Alternatively, if cell survival studies are being employed, a further blood sample can be taken from the patient at a fixed time after the radiolabelled cells have been injected and radioactive counts measured to provide an index of cell survival.

As mentioned hereinbefore the labelling of different species of blood cell provides a different diagnostic use. Accordingly radiolabelled metal(III) complexes of the present invention can with advantage be used to label granulocytes, platelets, red cells

and lymphocytes. Radiolabelled leucocytes can be used clinically to detect sites of abscess and inflammation. Radiolabelled platelets may be used diagnostically to measure platelet survival and distribution. Furthermore they may be used to visualize venous and arterial accumulation of platelets in atherosclerotic lesions, venous rhombi and thrombophlebitis. An additional use of radiolabelled platelets is to provide an early indication of renal transplant rejection and to assess the thrombogenicity of arterial grafts. The circulation and distribution of radiolabelled autologous lymphocytes can be visualized and analysed using the complexes of the present invention. Labelling of red blood cells is of diagnostic benefit in the following instances: cardiovascular imaging, gated wall motion studies, measurement of ejection function of the left ventricle, measurement of blood volume, gastro-intestinal bleeding studies and for the labelling of heat denatured red blood cells for splenic imaging.

In addition to the use of the complexes in diagnosis, lymphocytes labelled with radioactive indium complexes, for example of In 114, and potentially also gallium complexes, may be used in therapy, principally in the treatment of lymphomas. The complexes of the present invention are therefore also of interest in a therapeutic context.

The dosage of radiolabelled metal(III) complex labelled blood cell administered to the patient is dependent upon the radioactive isotope, the species of blood cell, the diagnostic purpose it is being employed for and the method of detection to be used. The following information may, however, be provided by way of guidance.

The normal adult dose of indium 111 labelled leucocytes for location of sites of infection is 500-1000 μCi (18.5 → 37 MBq). For indium(III) complexes of the present invention dosages within the range 300 to 1000 μCi, particularly 600 μCi, should generally be suitable.

For gallium(III) complexes of the present invention dosages within the range 300 to 1000, 2000 or 2500 μCi, particularly 500, 1000 or 1500 μCi should generally be suitable. Thus the normal adult dosage of radioactive gallium 67 is 1000-2000 μCi (37-74 MBq) for detecting tumours in the lymphatic system. For tumour visualization by scanning techniques a dosage of 1500-2500 μCi (55.5-92.5 MBq) is recommended.

The dosages used for indium 111 and gallium 67 complexes in other labelling contexts will be similar to those given above subject to any variations generally recognised in the literature as being suitable in that context. Moreover dosages for other radioactive isotopes will be similar in terms of μCi although not necessarily in terms of the amount of the complex.

Where the indium(III) or gallium(III) complexes are to be used in vivo either diagnostically or therapeutically, then these are best formulated into pharmaceutical compositions with a suitable diluent or carrier as described in UK Patents Nos. 2,117,766, 2,128,998 or 2,136,806.

The labelling of the desired blood cells with the complexes of the present invention can be conveniently achieved by methods described in the literature for other complexes which are illustrated in Examples 3 and 4.

The invention is illustrated by the following Examples, Examples 1 to 3 of which relate to intermediates for the preparation of the complexes. Certain of these intermediates are novel per se and are included within the scope of the present invention. The nmr signals have been attributed to the protons to which they are believed to relate.

EXAMPLES

Example 1 : Preparation of 3-hydroxy-6-methoxymethyl-4-pyrone

(A)

(1) 3-Benzyl-6-methoxymethyl-4-pyrone

3-Benzyloxy-6-hydroxymethyl-4-pyrone was prepared by heating at 60°C a mixture of 3-hydroxy-6-hydroxymethyl-4-pyrone and a 1.5 molar excess of benzyl chloride with a 1.5 molar excess of potassium carbonate in dimethylformamide for 24 hours. To the resulting solution of 3-benzyloxy-6-hydroxymethyl-4-pyrone (10 g, 0.023 mol) in dimethyl formamide was added iodomethane (14 ml, 0.215 mol) and the solution stirred for half an hour. Sodium hydride (2.6 g, 0.086 mol) was added and the mixture stirred for 24 hours under nitrogen gas. The dimethylformamide was then evaporated and the residue dissolved in water. The pH was then adjusted to pH 12 using 10M NaOH before extraction into dichloromethane (3 x 150 ml). The dichloromethane extracts were combined, dried over anhydrous sodium sulphate and evaporated to dryness. This yielded a yellow solid which was recrystallized from hot ethanol to provide the title compound (5.8 g, 55%).

(2) 3-Hydroxy-6-methoxymethyl-4-pyrone

3-Benzyl-6-methoxymethyl-4-pyrone (8 g, 0.032 mol) was refluxed in 100 ml hydrochloric acid for 2 hours. After cooling, the pH of the mixture was adjusted to pH 11, followed by extraction into dichloromethane (2 x 50 ml). The aqueous layer was treated with concentrated hydrochloric acid to pH 2 and further extracted into dichloromethane (3 x 100 ml). The extracts were combined, dried over anhydrous sodium sulphate and evaporated to dryness. This yielded 3.5 g (70%) of a white solid that was recrystallized from toluene to provide the title compound; melting point 79-80°C; ν_{max} (nujol) 1650, 1630, 1595, 1260, 1220, 1150, 950 cm^{-1}; δH (d^6-DMSO) 9.5 (1H, sbr, OH), 8.1 (1H, s, 2-H), 6.4 (1H, s, 5-H), 4.3 (2-H, s, CH_2), 3.3 (3H, s, CH_3).

(B) Other 3-hydroxy-6-alkoxymethyl-4-pyrones were prepared by the same method as described above using a five molar excess of the appropriate alkyl halide in step (1).

6-Ethoxymethyl-3-hydroxy-4-pyrone

Yield 8.2 g (73.3%) in step (1) and 4.2 g (80%) in step (2); Melting point 73-74°C; ν_{max} (nujol) 1650, 1610, 1265, 1220, 1100, 900 cm^{-1};

δH (d^6-DMSO), 9.15 (1H, sbr, OH), 8.1 (1H, s, 2-H), 6.4 (1H, s, 5-H), 4.3 (2H, s, CH_2), 3.4 (2H, q, $\underline{CH_2}CH_3$), 1.1 (3H, t, CH_3).

3-Hydroxy-6-propoxymethyl-4-pyrone

Yield 8.0 g (68%) in step (1) and 4.6 g (68.5%) in step (2); Melting point 63-64°C;

ν_{max} (nujol) 1650, 1620, 1580, 1265, 1210, 1150, 950 cm^{-1};

δH (d^6-DMSO), 9.1 (1H, b, OH), 8.1 (1H, s, 2-H), 6.4 (1H, s, 5-H), 4.3 (2H, s, CH_2), 3.4 (2H, b, $\underline{CH_2}CH_2CH_3$), 1.5 (2H, m, $CH_2\underline{CH_2}CH_3$), 0.9 (3H, t, CH_3).

6-Butoxymethyl-3-hydroxy-4-pyrone

Yield 8.3 g (83%) in step (1) and 5.4 g (78.6%) in step (2); Melting point 34-35°C;

ν_{max} (nujol) 1650, 1630, 1300, 1210, 1100, 920 cm^{-1};

δH (d^6-DMSO), 9.1 (1H, s, OH), 8.1 (1H, s, 2-H), 6.4 (1H, s, 5-H), 4.3 (2H, s, CH_2), 3.4 (2H, t, $\underline{CH_2}CH_2CH_2CH_3$), 1.4 (4H, m, $CH_2\underline{CH_2}\underline{CH_2}CH_3$), 0.9 (3H, t, CH_3).

Example 2 : Preparation of 3-hydroxy-2-hydroxymethyl-6-methyl-4-pyrone and other 3-hydroxy-2-(1-hydroxyalkyl)-6-methyl-4-pyrones (A)

(1) 3-Hydroxy-6-chloromethyl-4-pyrone

3-Hydroxy-6-hydroxymethyl-4-pyrone (50 g, 0.35 mol) was dissolved in 500 ml redistilled thionyl chloride and left to stand for 2 hours. The title compound was precipitated from the solution, filtered and washed in petroleum ether.

Recrystallisation from H_2O gave a yield of 41.6 g (74%);

δH (d^6-DMSO), 9.3 (1H, s, OH), 8.1 (1H, s, 3H), 6.6 (1H, s, 5-H), 4.7 (2H, s, CH_2).

(2) 3-Hydroxy-6-methyl-4-pyrone

3-Hydroxy-6-chloromethyl-4-pyrone (20 g, 0.125 mol) was added to 150 ml H_2O and heated to 40°C. Zinc dust (16.29 g, 0.25 mol) was added and the mixture stirred vigorously at 60°C. Concentrated hydrochloric acid (37 ml, 3 molar equivalent) was added dropwise over a period of one hour. The slurry was left stirring for 2-3 hours at 60°C. The excess zinc was removed by hot filtration and the pH of the filtrate adjusted to 1 and extracted into dichloromethane (3 x 100 ml). The combined extracts were dried under anhydrous sodium sulphate and evaporated to dryness to give the title compound (12.9 g, 86%). Recrystallisation was carried out from hot propanol, melting point 152-153°C;
ν_{max} (nujol) 1650, 1610, 1575, 1220, 1150, 920 cm^{-1};
δH (d^6-DMSO), 8.9 (1H, s, OH), 8.0 (1H, s, 2-H), 6.3 (1H, s, 5-H), 2.2 (3H, s, CH_3).

(3) 3-Hydroxy-2-hydroxymethyl-6-methyl-4-pyrone

3-Hydroxy-6-methyl-4-pyrone (9.4 g, 0.074 mol) was added to 100 ml H_2O. Formaldehyde (5.5 ml, 0.074 mol) was added and the final pH of the mixture adjusted to 10.5. The solution was stirred at 25°C for 24 hours. The pH ws then adjusted to 1 and the title compound precipitated (10 g, 86.2%);
δH (d^6-DMSO), 8.8 (1H, sbr, OH), 6.2 (1H, s, 5-H),
5.7 (1H, sbr, $CH_2\underline{O}H$), 4.4 (2H, d, $\underline{CH_2}OH$), 2.3 (3H, s, CH_3).

(B) Using the appropriate aldehyde in a 1:1 molar ratio to the 3-hydroxy-6-methyl-4-pyrone in step (3) above the following 2-hydroxyalkyl compounds were prepared.

3-Hydroxy-2-(1-hydroxyethyl)-6-methyl-4-pyrone
δH (d^6-DMSO), 8.7 (1H, sbr, OH), 6.2 (1H, s, 5-H),
5.4 (1H, sbr, $CH\underline{O}H$), 5.0 (1H, dbr, $\underline{CH}$), 2.3 (3H, s, CH_3),
1.4 (3H, d, $\underline{CH_3}CH$).

3-Hydroxy-2-(1-hydroxypropyl)-6-methyl-4-pyrone
δH (d^6-DMSO), 8.7 (1H, sbr, OH), 6.2 (1H, s, 5-H),
5.3 (1H, d, $\underline{CH}$-OH), 4.7 (1H, m, $\underline{CH}$-OH), 2.3 (3H, s, CH_3),
1.5 (2H, m, CH_2), 0.9 (3H, t, $CH_2\underline{CH_3}$).

Example 3 : Preparation of 2,6-dimethyl-3-hydroxy-4-pyrone and other 2-alkyl-6-methyl-4-pyrones

(A) 3-Hydroxy-2-hydroxymethyl-6-methyl-4-pyrone (prepared as described in Example 2) (2 g, 0.013 mol) was dissolved in 100 ml H_2O. Zinc dust (1.7 g, 0.026 mol) was added and the mixture was stirred vigorously at 60°C. Concentrated HCl (13 ml, 10 molar equivalent) was added dropwise over a period of about an hour. The slurry was left stirring at 60°C for 12 hours. The excess zinc was then removed by hot filtration, the pH was adjusted to 1 and the mixture was extracted into dichloromethane (3 x 50 ml). The extracts were combined, dried over anhydrous sodium sulphate and evaporated to dryness to yield the title compound (0.8 g, 47%). This was recrystallized from toluene, melting point 163-164°C;

ν_{max} (nujol) 1660, 1630, 1580, 1220, 1080, 970, 930 cm^{-1};

δH (d^6-DMSO), 8.5 (1H, sbr, OH), 6.2 (1H, s, 5-H), 2.2 (6H, s, CH_3CH_3).

(B) Other 2-alkyl-6-methyl-4-pyrones may be prepared in a similar manner from the corresponding 3-hydroxy-2-(1-hydroxyalkyl-6-methyl-4-pyrones.

Example 4 : Preparation of 3:1 ligand:indium(III) and gallium(III) complexes

(A) Preparation of (ethyl-maltol)$_3$-gallium 67 complex

Ethyl maltol is prepared as described in Example 1 of U.S. Patent Application 310,141 referred to in U.S. Patent No. 3,376,317. The ethyl maltol is made up in stock solutions at a concentration of 0.01M in 20 mM HEPES/0.8.% NaCl, pH 7.4-7.5. The more concentrated stock solution of ethyl-maltol is added dropwise to an acidic solution of ^{67}Ga-($GaCl_3$) in 0.04 N HCl. The resulting solution is neutralized to pH 7.0.

Complexes of other substituted 3-hydroxy-4-pyrones are prepared in a similar manner.

(B) Preparation of (1-ethyl-3-hydroxypyridin-2-one)$_3$-indium III complex

The synthesis of 1-ethyl-3-hydroxypyridin-2-one is described in Example 1 of European Patent No. 094149.

The 1-ethyl-3-hydroxypyridin-2-one is made up into stock solutions in 20 mM HEPES/0.8 NaCl pH 7.4-7.5 at a final concentration of 0.01M. The 1-ethyl-3-hydroxypyridin-2-one solution is added dropwise to an acidic solution of ^{111}In-($InCl_3$) in 0.04 N HCl. The resulting solution is neutralized to pH 7.0.

Complexes of other 3-hydroxypyridin-2-ones are prepared in a similar manner.

In both the case of the hydroxypyrones and hydroxypyridinones the ligands are preferably added in large excess as compared with the metal salt, for example a 100:1 or 1000:1 rather than a 3:1 molar proportion of ligand providing compound:salt, in order to ensure that all the radioactive metal cations combine with the ligand providing compound in the form of the 3:1 complex.

Example 5 : Partition coefficients

Partition coefficients of complexes were determined by addition of 1 ml of a solution of the ^{67}Ga-(III) and ^{111}In-(III) 3:1 complex prepared as described in Example 1 using a 3:1 molar proportion to 1 ml of water-saturated octan-1-ol in a glass test tube. The system was allowed to equilibrate at ambient temperature for 15 minutes on a gently rotating mixer. The tubes were spun at 3000 rpm (1500 g) for 10 minutes after which time 200 microliter samples of each phase were taken, weighed using an analytical balance and counted for radioactivity. Partition coefficients were calculated using the following equation:

$$\text{p.c.} = \frac{\text{counts per minute/gram octanol}}{\text{counts per minute/gram HEPES/saline}}$$

The partition coefficients obtained for various 3-hydroxy-4-pyrone (HP) complexes are shown in Table 1.

TABLE 1

COMPOUND	PARTITION COEFFICIENT (K_{part})	
	^{67}Ga	^{111}In
maltol	0.17	1.33 (±0.04)
ethyl-maltol	4.22	18.19 (±1.17)
isopropyl-maltol	20.9	-
butyl-maltol	72.5	-

The partition coefficients of various ligand-providing compounds described in Examples 1 to 3 were also determined.

Solutions of the ligands were produced by dissolving the ligand in aqueous tris hydrochloride of pH 7.4. Acid washed glassware was used throughout and, following mixing of 5 ml of the 10^{-4}M aqueous solution with 5 ml of n-octanol for 1 minute, the aqueous n-octanol mixture was centrifuged at 1,000 g for 30seconds. The two resulting phases were separated for a concentration determination by spectrophotometry on each. Values typical of those obtained are shown in Table 2.

TABLE 2

COMPOUND	PARTITION COEFFICIENT (K_{part})
3-hydroxy-6-methyl-4-pyrone	0.261 ± 0.013
3-hydroxy-2,6-dimethyl-4-pyrone	2.766 ± 0.034
2-(trifluoromethyl)-3-hydroxy-4-pyrone	0.639 ± 0.136
3-hydroxy-2-methoxymethyl-4-pyrone	0.238 ± 0.047
2-ethoxymethyl-3-hydroxy-4-pyrone	0.417 ± 0.062
3-hydroxy-2-propoxymethyl-4-pyrone	2.004 ± 0.182
2-butoxymethyl-3-hydroxy-4-pyrone	5.992 ± 0.460

Example 6 : Red cell labelling with [^{67}Ga(III)-(3-hydroxy-4-pyrone)$_3$] complexes

Red cell labelling was performed using whole blood taken from normal adult human volunteers. The blood was initially spun at 1500 rpm (400 g) for 15 minutes and the plasma removed. The packed cells were washed with an equal volume of heparinised saline, centrifuged to remove the saline and the wash step repeated. The washed packed cells were pooled and briefly rotated on a mixer-rotator before use. For cell labelling experiments 1 ml of washed cells was added to a heparinised glass blood collection tube. To this 1 ml of cells was added 0.5 ml of a solution of the ^{67}Ga(III) 3:1 3-hydroxy-4-pyrone complex prepared as described in Example 1 using a 100:1 to 1000:1 molar proportion. The tubes were capped and placed on a rotating mixer for the duration of the study, except for brief sampling when required. One hundred microliter samples of the cell suspensions were taken at various time intervals and added to clean glass test tubes containing 2 ml of saline. The sample suspension was mixed well and centrifuged at 3000 rpm (1500 g) for 5 minutes. The supernatant was removed and the cells washed for a second time with saline. After removal of the second saline wash the cells were lysed using deionised water and the final volume made up to match that of the previous saline washes. Both cells and supernatant were counted using an auto gamma counter set for ^{67}Ga photon energies (95-300 keV).

The results are illustrated in the accompanying Figures 1 to 5. Each figure shows the percentage of ^{67}Ga-complex incorporated into the red blood cells as a function of time,

Figure 1 represents the data for ^{67}Ga(III)-(maltol)$_3$ for comparative purposes,

Figure 2 represents the data for ^{67}Ga(III)-(ethyl-maltol)$_3$,

Figure 3 for ^{67}Ga(III)-(isopropyl-maltol)$_3$, and

Figure 4 for ^{67}Ga(III)-(butyl-maltol)$_3$.

As can be seen from these figures the complexes containing the larger alkyl groups allow for more rapid uptake of ^{67}Ga into the red cells and achieve a higher absolute incorporation.

Figure 5 shows the effect of ligand concentration on uptake of ^{67}Ga into erythrocytes at 15 minutes for various complexes. The graphs labelled 1 to 4 represent, respectively, the results for the maltol, ethyl-maltol, isopropyl-maltol and butyl-maltol ^{67}Ga complexes (the first for comparative purposes). Again this indicates that the complexes containing the larger alkyl groups give increased incorporation of ^{67}Ga into red cells.

Example 7 : Leucocyte labelling with [^{111}In(III)-(3-hydroxy-4-pyrones)$_3$]

The effect of ligand concentration on white cell labelling with $^{111}In(III)$ 3:1 3-hydroxy-4-pyrone complexes prepared as described in Example 1 using a 100:1 to 1000:1 molar proportion was performed using mixed whited cell populations suspended in buffer. The white cells were harvested from 60 ml of whole blood obtained from normal human volunteers. The blood was drawn into a 60 ml syringe, anticoagulated using heparin (100 Units/10 ml whole blood) and the red cells allowed to sediment by standing the syringe upright using a ring-stand for 1 hour. At the end of 1 hour the majority of the white cells were found in the supernatant plasma. The supernatant was expressed into a sterile plastic tube using a 19 g bufferfly set. The cells were washed twice using 20 mM HEPES-saline (0.8%) and resuspended in 5 ml buffer. A cell count and differential was performed using a coulter-counter device and the cell suspension concentration was adjusted to 9×10^6 white cells/0.9 ml. Labelling was performed by adding 0.1 ml of a solution of the appropriate $^{111}In(III)$ 3:1 complex and incubating for 15 minutes. After 15 minutes incubation the cells were washed twice with 2 ml of buffer. The cells and separate washes were counted using an automatic gamma counter with energy windows set for ^{111}In.

Figure 6 shows the effect of ligand concentration on complex incorporation into leucocytes, the labelling of the graphs being as indicated above for Figure 5. These results are similar to those observed for the gallium complexes in Example 3 in that increased incorporation is obtained with the complexes containing the larger alkyl groups.

Example 8 : The effect of cell concentration on radiolabelled complex incorporation

The effect of white cell concentration was studied using radiolabelled ^{111}In-(III)-(2-butyl-3-hydroxypyridin-2-one)$_3$ complex. Cells were labelled, separated and counted using the procedures of Example 4. The effect of red cell volume was investigated using the butyl-maltol 3:1 Ga(III) complex. 0.5 ml of the complex prepared as in Example 1 using a 100:1 to 1000:1 molar proportion was added to heparinised glass blood collection tubes containing 1, 2, 3, 4 or 5 ml of washed, packed red cells obtained as in Example 3. 100 µl aliquots of cell suspension were removed at time 5, 15, 30 and 45 minutes, added to tubes containing 2 ml of 0.9% saline and washed, separated and counted using the procedures of Example 3.

Figure 7 shows the effect of leucocyte concentration on indium labelled complex incorporation at 0.0001 M concentration of the butyl-maltol 3:1 ^{11} In(III) complex, incubation being for 15 minutes. Cell concentration had no significant effect on percentage uptake of the radiolabelled complex. These results were paralleled in the red cell volume studies for gallium labelled complex incorporation of the butyl-maltol 3:1 ^{67}Ga(III) complex (results not shown).

CLAIMS

1. A neutral 3:1 ligand:metal(III) complex, in which the trivalent metal cation is a radioactive isotope of indium or gallium and each ligand is separately provided by a compound being:

(1) a 3-hydroxy-4-pyrone in which one or more of the hydrogen atoms attached to ring carbon atoms is replaced by an aliphatic hydrocarbon group of one to six carbon atoms or such a group substituted by one or more groups selected from fluoro, hydroxy and aliphatic hydrocarbyloxy groups but excluding 3-hydroxy-2-methyl-4-pyrone; or

(2) 3-hydroxypyridin-2-one or a 3-hydroxypyridin-2-one in which the hydrogen atom attached to the nitrogen atom is replaced by an aliphatic acyl group, by an aliphatic hydrocarbon group of 1 to 6 carbon atoms, or by an aliphatic hydrocarbon group substituted by one or more substituents selected from aliphatic acyl, alkoxy, cycloalkoxy, aliphatic amide, aliphatic ester, halogen and hydroxy groups and optionally, in which one or more of the hydrogen atoms attached to ring carbon atoms is replaced by one of said substituents, by an aliphatic hydrocarbon group of 1 to 6 carbon atoms, or by an aliphatic hydrocarbon group substituted by an alkoxy, cycloalkoxy, aliphatic ester or hydroxy group or by one or more halogen groups.

2. A neutral 3:1 ligand:metal(III) complex, in which the trivalent metal cation is a radioactive isotope of indium or gallium and each ligand is separately provided by a compound being:

(1) a 3-hydroxy-4-pyrone in which one or more of the hydrogen atoms attached to ring carbon atoms is replaced by an aliphatic hydrocarbon group of one to six carbon atoms or such a group substituted by one or more fluoro groups but excluding 3-hydroxy-2-methyl-4-pyrone; or

(2) 3-hydroxypyridin-2-one or a 3-hydroxypyridin-2-one in which the hydrogen atom attached to the nitrogen atom is replaced by an aliphatic acyl group, by an aliphatic hydrocarbon group of 1 to 6 carbon atoms, or by an aliphatic hydrocarbon group substituted by one or more substituents selected from aliphatic acyl, alkoxy,

cycloalkoxy, aliphatic amide, aliphatic ester, halogen and hydroxy groups and optionally, in which one or more of the hydrogen atoms attached to ring carbon atoms is replaced by one of said substituents, by an aliphatic hydrocarbon group of 1 to 6 carbon atoms, or by an aliphatic hydrocarbon group substituted by an alkoxy, cycloalkoxy, aliphatic ester or hydroxy group or by one or more halogen groups.

3. A complex according to Claim 1 or 2, in which the trivalent metal cation is of In^{111} or In^{113}.

4. A complex according to Claim 1 or 2, in which the trivalent metal cation is of Ga^{67} or Ga^{68}.

5. A complex according to any of the preceding claims, in which each ligand separately is provided by a compound selected from those of type (1).

6. A complex according to Claim 5, in which each ligand is provided by the same compound of type (1).

7. A complex according to any of the preceding claims, in which the compound of type (1) is a 3-hydroxy-4-pyrone in which one or more of the hydrogen atoms attached to ring carbon atoms is replaced by the same or different substituents selected from methyl, hydroxymethyl, methoxymethyl, ethoxymethyl, propoxymethyl, butoxymethyl, ethyl, 1-hydroxyethyl, 1,1,2,2,2-pentafluoroethyl, propyl, isopropyl, 1-hydroxypropyl, 3,3,3-trifluoropropyl, butyl, 1-hydroxybutyl and pentyl groups.

8. A complex according to Claim 7, in which the compound is 3-hydroxy-2,6-dimethyl-4-pyrone, 2-ethyl-3-hydroxy-4-pyrone, 2-(1',1',2',2',2'-pentafluoroethyl)-3-hydroxy-4-pyrone, 3-hydroxy-2-propyl-4-pyrone, 3-hydroxy-2-(1'-methylethyl)-4-pyrone, 2-(3',3',3'-trifluoropropyl)-3-hydroxy-4-pyrone or 2-butyl-3-hydroxy-4-pyrone.

9. A complex according to Claim 7, in which the compound is 3-hydroxy-6-methoxymethyl-4-pyrone, 6-ethoxymethyl-3-hydroxy-4-pyrone, 3-hydroxy-6-propoxymethyl-4-pyrone or 6-butoxymethyl-3-hydroxy-4-pyrone.

10. A complex according to Claim 7, in which the compound is 2-ethyl-3-hydroxy-6-methyl-4-pyrone, 3-hydroxy-6-methyl-2-propyl-4-pyrone or 2-butyl-3-hydroxy-6-methyl-4-pyrone.

11. A complex according to Claim 7, in which the compound is 3-hydroxy-2-hydroxymethyl-6-methyl-4-pyrone, 3-hydroxy-2-(1-hydroxyethyl)-6-methyl-4-pyrone, 3-hydroxy-2-(1-hydroxypropyl)-6-methyl-4-pyrone or 3-hydroxy-2-(1-hydroxybutyl)-6-methyl-4-pyrone.

12. A complex according to any of Claims 1 to 4, in which each ligand separately is provided by a compound selected from those of type (2).

13. A complex according to Claim 12, in which each ligand is provided by the same compound of type (2).

14. A complex according to any of Claims 1, 2, 3, 4, 12 and 13, in which the compound of type (2) is 3-hydroxypyridin-2-one in which the hydrogen attached to the nitrogen is replaced by an aliphatic hydrocarbon gorup of 1 to 6 carbon atoms or such a group substituted by one or more fluoro groups and, optionally, in which one or more of the hydrogen atoms attached to ring carbon atoms is replaced by the same or a different group which is an aliphatic hydrocarbon group of 1 to 6 carbon atoms or such a group substituted by one or more fluoro groups.

15. A complex according to Claim 14, in which the aliphatic hydrocarbon groups are selected from methyl, ethyl, 2,2,2,3,3-pentafluoroethyl, n-propyl, isopropyl, 3,3,3-trifluoropropyl, and straight and branched chain butyl and pentyl groups.

16. A complex according to Claim 15, in which the compound is 1-ethyl-3-hydroxypyridin-2-one, 1-(1',1',2',2',2'-pentafluoroethyl)-3-hydroxypyridin-2-one, 3-hydroxy-1-propylpyridin-2-one, 3-hydroxy-1-(1'-methylethyl)-pyridin-2-one, 1-(3',3',3'-trifluoropropyl)-3-hydroxypyridin-2-one or 1-butyl-3-hydroxypyridin-2-one.

17. A neutral 3:1 ligand:metal(III) complex according to any of the Claims 1 to 16 for use in diagnosis or therapy.

18. A pharmaceutical composition comprising a neutral 3:1 ligand:metal(III) complex according to any of Claims 1 to 16, together with a physiologically acceptable diluent or carrier.

19. A method for the diagnosis of atherosclerosis, thrombocytopenia, venous thrombi or thrombophlebitis, which comprises radiolabelling red blood cells with a neutral 3:1 ligand:metal(III) complex according to any of Claims 1 to 16.

20. A method for the detection of abscesses, inflammation, tumour or metastatic sites and therapy thereof which comprises radiolabelling lymphocytes with a neutral 3:1 ligand:metal(III) complex according to any of Claims 1 to 16.

21. A method for assessing the thromogenicity of arterial grafts and for providing an early indication of renal transplant rejection comprising radiolabelling blood platelets with a neutral 3:1 ligand:metal(III) complex according to any of Claims 1 to 16.

22. A method of diagnosis utilising radioactively labelled red blood cells for cardiovascular imaging, gated wall motion studies, measurement of ejection function of the left ventricle, measurement of blood volume, gastro-intestinal bleeding and splenic imaging comprising radiolabelling red blood cells with a neutral 3:1 ligand:metal(III) complex according to any of Claims 1 to 16.

23. A 3-hydroxy-4-pyrone in which one or more of the hydrogen atoms attached to the ring carbon atoms is replaced by an aliphatic hydrocarbon group of one to six carbon atoms substituted by an aliphatic hydrocarbyloxy group of one to six carbon atoms and one or more other atoms are optionally replaced by an aliphatic hydrocarbon group of one to six carbon atoms.

24. A 3-hydroxy-4-pyrone according to Claim 23 being 3-hydroxy-6-methoxymethyl-4-pyrone, 6-ethoxymethyl-3-hydroxy-4-pyrone, 3-hydroxy-6-propoxymethyl-4-pyrone or 6-butoxymethyl-3-hydroxy-4-pyrone.

25. A 3-hydroxy-6-methyl-4-pyrone substituted at the 2-position by a C_{1-6} aliphatic hydrocarbon group or such a group substituted by a hydroxy group but excluding 3-hydroxy-2,6-dimethyl-4-pyrone.

26. A 3-hydroxy-6-methyl-4-pyrone according to Claim 25 being 2-ethyl-3-hydroxy-6-methyl-4-pyrone, 3-hydroxy-6-methyl-2-propyl-4-pyrone or 2-butyl-3-hydroxy-6-methyl-4-pyrone.

27. A 3-hydroxy-6-methyl-4-pyrone being 3-hydroxy-2-hydroxymethyl-6-methyl-4-pyrone, 3-hydroxy-2-(1-hydroxyethyl)-6-methyl-4-pyrone, 3-hydroxy-2-(1-hydroxypropyl)-6-methyl-4-pyrone or 3-hydroxy-2-(1-hydroxybutyl)-6-methyl-4-pyrone.

28. A process for the preparation of a complex according to Claim 1 which comprises reacting the corresponding 3-hydroxy-4-pyrone and/or 3-hydroxypyrid-2-one ligand-providing compound or compounds with the trivalent cation of the indium or gallium isotope.

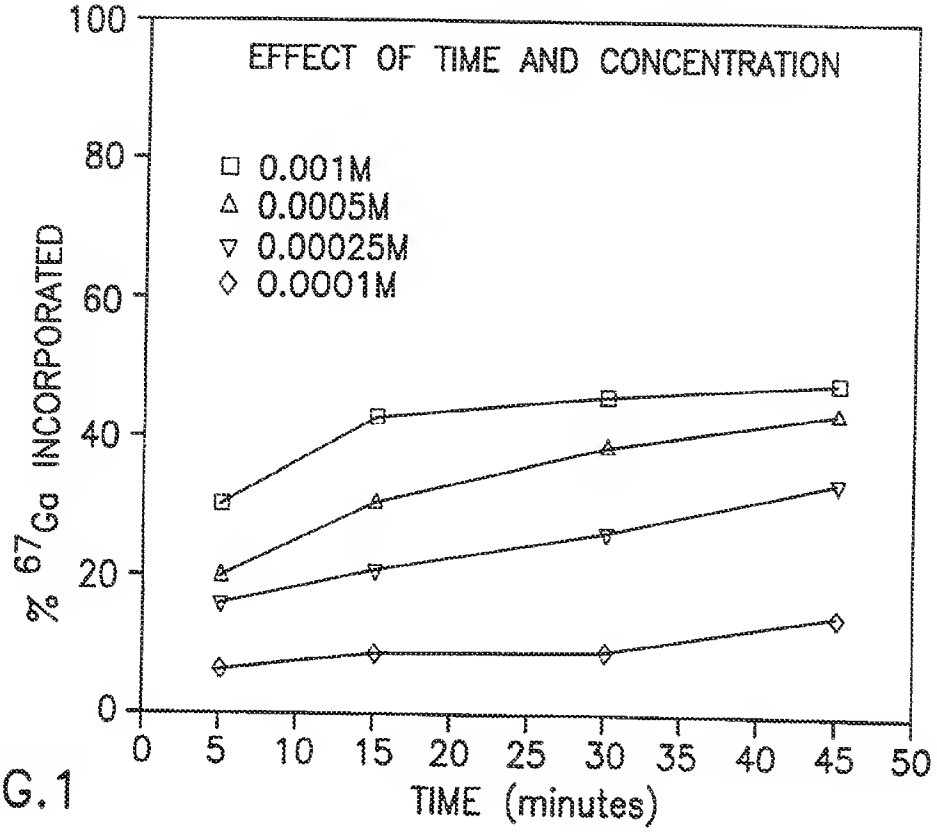
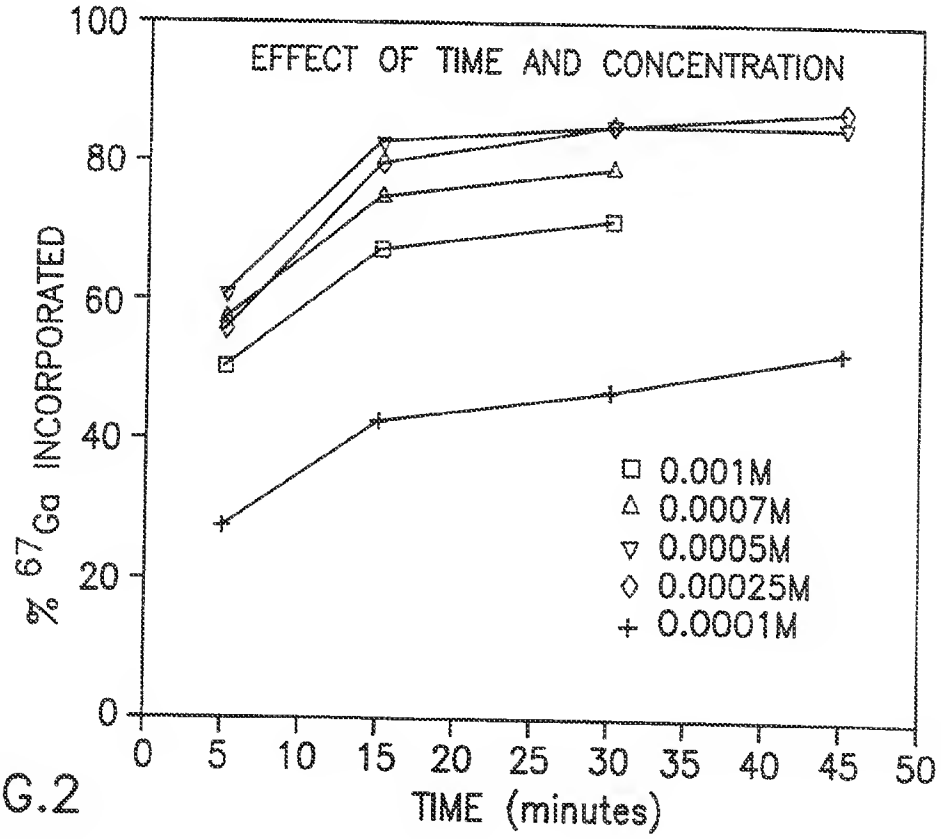

EFFECT OF TIME AND CONCENTRATION
0.001M
0.0005M
0.00025M
0.0001M
% 67Ga INCORPORATED
TIME (minutes)
0
20
40
60
80
100
0 5 10 15 20 25 30 35 40 45 50

FIG.1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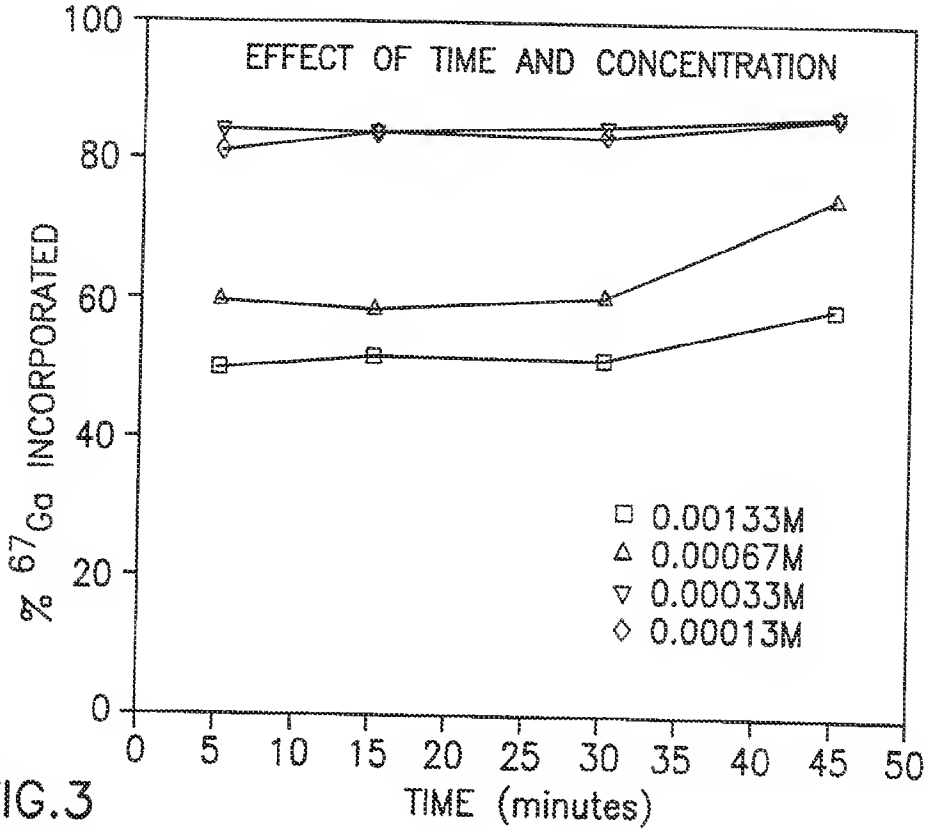
0.0007M
0.0005M
0.00025M
0.0001M
% 67Ga INCORPORATED
TIME (minutes)
0
20
40
60
80
100
0 5 10 15 20 25 30 35 40 45 50

FIG.2


EFFECT OF TIME AND CONCENTRATION
% 67Ga INCORPORATED
TIME (minutes)
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0.00067M
0.00033M
0.00013M
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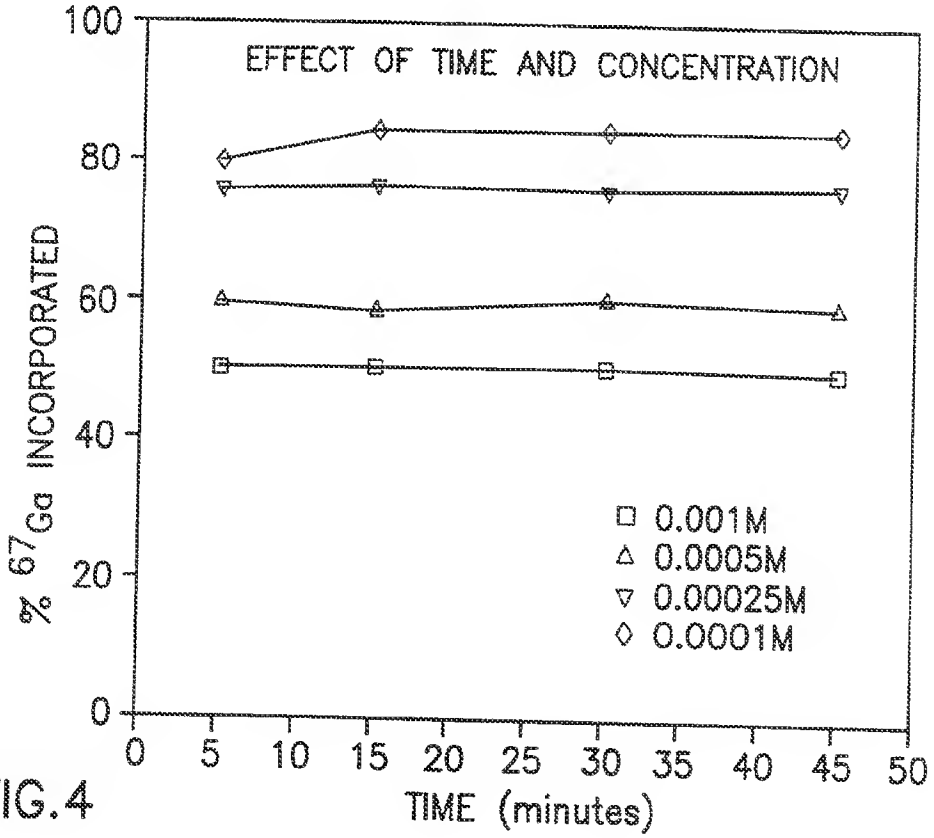
60
80
100
0
5
10
15
20
25
30
35
40
45
50

FIG.3


EFFECT OF TIME AND CONCENTRATION
% 67Ga INCORPORATED
TIME (minutes)
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0.0005M
0.00025M
0.0001M
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40
60
80
100
0
5
10
15
20
25
30
35
40
45
50

FIG.4

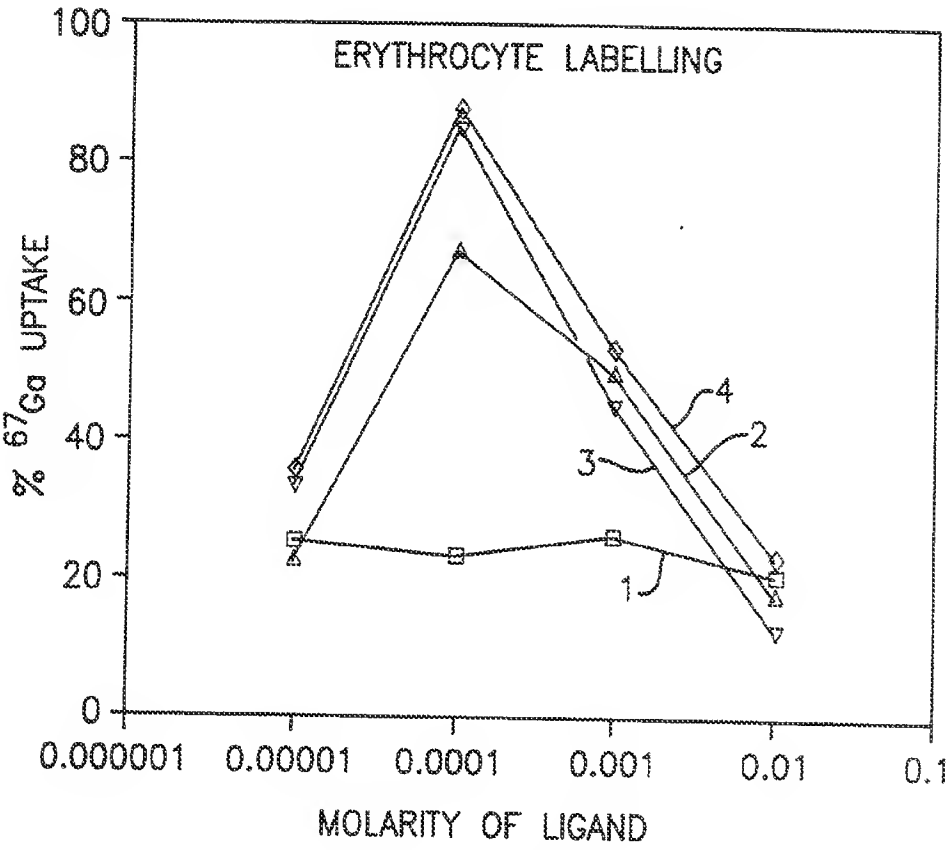
ERYTHROCYTE LABELLING
% 67Ga UPTAKE
100
80
60
40
20
0
0.000001
0.00001
0.0001
0.001
0.01
0.1
MOLARITY OF LIGAND
1
2
3
4

FIG.5

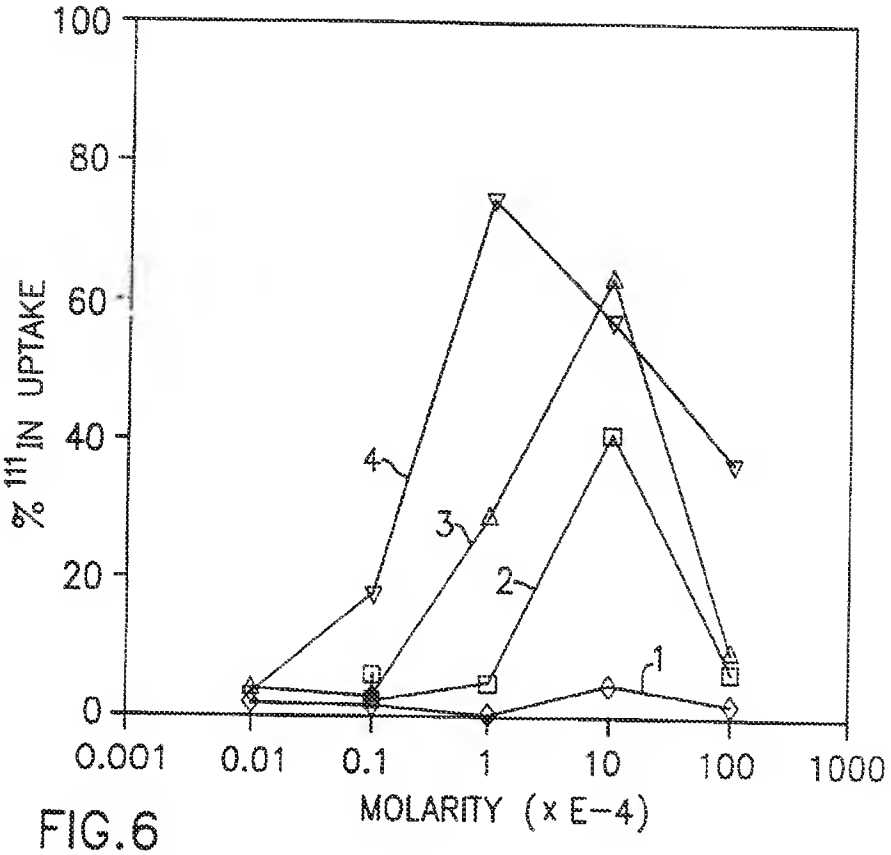
100
80
60
40
20
0
% 111IN UPTAKE
4
3
2
1
0.001
0.01
0.1
1
10
100
1000
MOLARITY (x E-4)

FIG.6

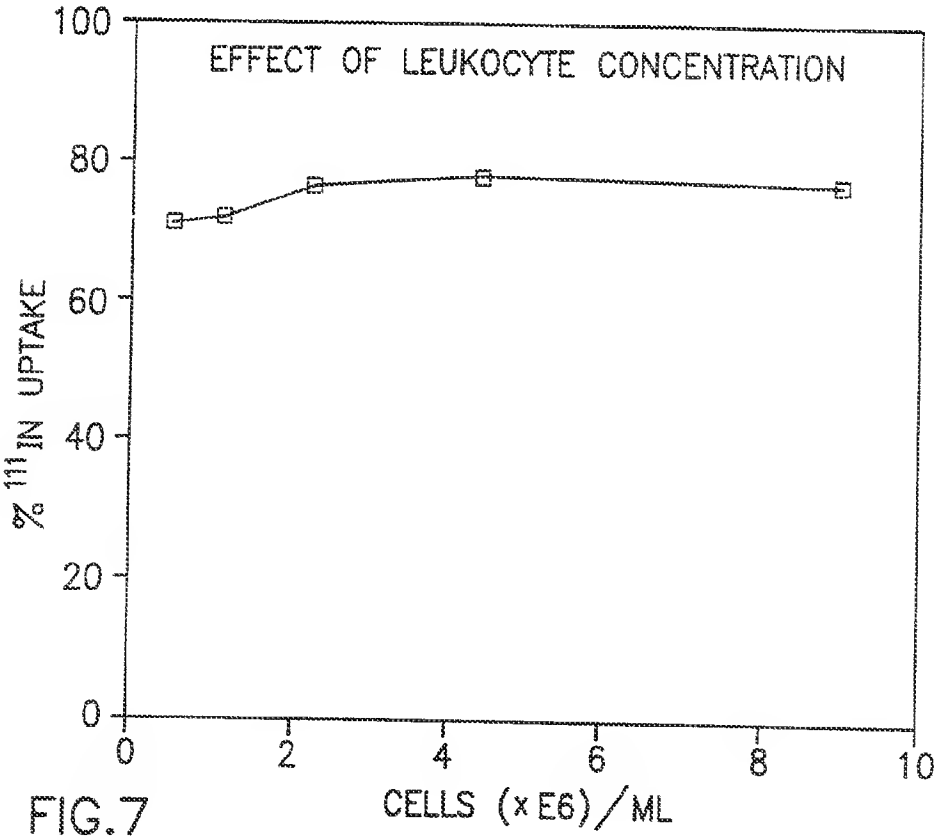
EFFECT OF LEUKOCYTE CONCENTRATION
100
80
60
40
20
0
% 111IN UPTAKE
0
2
4
6
8
10
CELLS (x E6)/ML

FIG.7

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 91/00441

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5]: C 07 B 59/00, C 07 D 309/40, C 07 F 5/00

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[5]	C 07 B 59/00, C 07 D 309/00, C 07 F 5/00

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	FR, A, 1516463 (PFIZER) 8 March 1968 see examples III,IV,XI; page 6, column 1, lines 12-16; résumé ---	25-27
X	Journal of Organic Chemistry, volume 43, no. 14, 7 July 1978, American Chemical Society, (Washington, DC, US), N.S. Poonia et al.: "Coordinative role of alkali cations in organic synthesis.3. Selective methylations of 5-hydroxy-2-hydroxymethyl-γ-pyrone", pages 2842-2844 see the whole article ---	23,24
A	Chemical Abstracts, volume 94, no.5, 2 February 1981, (Columbus, OH, US), J. Schuhmacher et al.: "Liver and kidney imaging with gallium-68-labeled dihydroxyanthraquinones", see page 233, abstract 26836t, & J. Nucl. Med., 1980, 21(10) 983-7 ---	1-18

./.

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
14th June 1991	20 AUG 1991
International Searching Authority: EUROPEAN PATENT OFFICE	Signature of Authorized Officer: MISS T. TAZELAAR

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	GB, A, 2117766 (NRDC) 19 October 1983 see examples cited in the application	1-18
A	GB, A, 2136806 (NRDC) 26 September 1984 see examples 2-4 cited in the application	1-18
A	GB, A, 2128998 (NRDC) 10 May 1984 see examples cited in the application	1-18
A	Inorganic Chemistry, volume 26, no. 13, 1 July 1987, American Chemical Society, (Washington, DC, US), M.M. Finnegan et al.: "Neutral water-soluble post-transition-metal chelate complexes of medical interest: Aluminum and Gallium Tris(3-hydroxy-4-pyronates)", pages 2171-2176 see pages 2171,2172 cited in the application	1-18
A	Inorganic Chemistry, volume 27, no. 22, 2 November 1988, American Chemical Society, (Washington, DC, US), C.A. Matsuba et al.: "Neutral water-soluble Indium complexes of 3-hydroxy-4-pyrones and 3-hydroxy-4-pyridinones", pages 3935-3939 see pages 3935,3936 cited in the application	1-18

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

A	EP, A, 0131327 (MALLINCKRODT) 16 January 1985 see the claims ---	1-18
A	FR, A, 2390408 (COMPAGNIE FRANCAISE PHILIPS) 8 December 1978 see the claims ---	1-18
A	GB, A, 1423482 (BYK-MALLINCKRODT CHEMISCHE PRODUKTE) 4 February 1976 see the whole document ---	1-18
	./.	

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers **, because they relate to subject matter not required to be searched by this Authority, namely:

** Claim numbers 19-22. Please see Rule 39.1(iv): methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods

2. [ ] Claim numbers ....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claim numbers ....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 9100441 SA 46020

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 08/08/91
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
FR-A- 1516463		GB-A-	1173383	10-12-69
		US-A-	3468915	23-09-69
EP-A- 0131327	16-01-85	AU-B-	580958	09-02-89
		AU-A-	2984584	10-01-85
		CA-A-	1234757	05-04-88
		JP-A-	60069040	19-04-85
		US-A-	4705678	10-11-87
FR-A- 2390408	08-12-78	None		
GB-A- 1423482	04-02-76	DE-A-	2304381	01-08-74
		CH-A-	588866	15-06-77
		FR-A,B	2215237	23-08-74
		NL-A-	7401191	01-08-74
GB-A- 2117766	19-10-83	EP-A,B	0093498	09-11-83
		EP-A,B	0094149	16-11-83
		GB-A,B	2118176	26-10-83
		JP-A-	58174318	13-10-83
		JP-A-	58174319	13-10-83
		US-A-	4550101	29-10-85
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		JP-A-	59181258	15-10-84
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		US-A-	4834983	30-05-89
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